IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Noriyuki KIZAKI et al.

Title: NOVEL CARBONYL REDUCTASE, GENE THEREOF AND METHOD OF USING THE SAME

Appl. No.: Unassigned

Filing Date: 03/22/2002

Examiner: Unassigned

Art Unit: Unassigned

PRELIMINARY AMENDMENT

Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the following amendments be entered into the application:

In the Specification:

On page 20, delete the 2nd full paragraph, and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

(3) Optimum action temperature:

The enzyme activity against the substrate N-benzyl-3-pyrrolidinone exerted for one minute of the reaction was measured within the temperature range of 20°C to 60°C. As a result, the optimum temperature was found to be 40°C to 45°C.

On page 27, delete the 3rd full paragraph, and replace this paragraph with the following in accordance with 37 CFR §1.121. A marked up version showing changes is attached:

Example 9: BRD expression in recombinant E.coli)

The recombinants E.coli HB101 (pTBH) and HB101 (PTSBH) obtained in Example 8 were each shake-cultured on 2 X YT medium containing 200 g/m1 of ampicillin at 28°C for 15 hours. A 1-ml portion of this preculture fluid was inoculated into 100 ml of a medium sterilized by autoclaving in a 500-ml Sakaguchi flask and comprising 1.5% (w/v) glycerol, 1.5% (w/v) Bacto tryptone, 0.4% (w/v) Bacto yeast extract, 0.2% (w/v) sodium chloride, 0.8% (w/v) potassium dihydrogen Phosphate, 0.05% (w/v) magnesium sulfate heptahydrate and 0.033% (w/v) Adekanol LG109 (product of Asahi Denka Kogyo), as adjusted to pH 6.0, and shake culture was carried out at 30°c for 60 hours. Cells were harvested from such culture fluids by using a centrifuge, then suspended in 100 mM phosphate buffer (pH 6.5) and untrasonically disrupted to give a cell-free exract.

In the Claims:

In accordance with 37 CFR §1.121, please substitute for original claims 3, 5, 8, 13, 14, 16 and 20 the following rewritten versions of the same claims, as amended. The changes are shown explicitly in the attached "Version With Markings to Show Changes Made."

3. (Amended) The polypeptide according to Claim 1 which is derived from a microorganism belonging to the genus Micrococcus.

5. (Amended) A DNA coding for the polypeptide according to Claim 1.

8. (Amended) An expression vector containing DNAs according to Claim 1.

13. (Amended) A transformant containing the expression vector according to Claim 1.

14. (Amended) A transformant containing both the expression vector according to Claim 1 and an expression vector containing a DNA coding for a polypeptide having glucose dehydrogenase.

16. (Amended) The transformant according to Claim 1, wherein a host thereof is Escherichia coli.

20. (Amended) A production method of (S)-N-benzyl-3-pyrrolidinol comprising

a step of reacting the transformant according to Claim 1 and/or a treated product thereof with N-benzyl-3-pyrrolidinone, and

a step of harvesting the thus-produced (S)-N-benzyl-3-pyrrolidinol.

REMARKS

Applicants respectfully request that the foregoing amendments to Claims 3, 5, 8, 13, 14, 16 and 20 be entered in order to avoid this application incurring a surcharge for the presence of one or more multiple dependent claims.

Respectfully submitted,

Date March 25, 2002

By _____

Harold C. Wegner
Attorney for Applicant
Registration No. 25,258

FOLEY & LARDNER
Customer Number: 22428

22428
PATENT TRADEMARK OFFICE

Telephone: (202) 672-5571
Facsimile: (202) 672-5399

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please amend the second full paragraph on page 20 as follows:

(3) Optimum action temperature:

The enzyme activity against the substrate N-benzyl-3-pyrrolidinone exerted for one minute of the reaction was measured within the temperature range of 20°C to 60°C. As a result, the optimum temperature was found to be 40°C to 45°C.

Please amend the third full paragraph starting on page 27 to page 28 as follows:

(Example 9: BRD expression in recombinant E.coli)

The recombinants E.coli HB101 (pTBH) and HB101 (PTSBH) obtained in Example 8 were each shake-cultured on 2 X YT medium containing 200 µg/m1 of ampicillin at 28°C for 15 hours. A 1-ml portion of this preculture fluid was inoculated into 100 ml of a medium sterilized by autoclaving in a 500-ml Sakaguchi [flaks] flask and comprising 1.5% (w/v) glycerol, 1.5% (w/v) Bacto tryptone, 0.4% (w/v) Bacto yeast extract, 0.2% (w/v) sodium chloride, 0.8% (w/v) potassium dihydrogen Phosphate, 0.05% (w/v) magnesium sulfate heptahydrate and 0.033% (w/v) Adekanol LG109 (product of Asahi Denka Kogyo), as adjusted to pH 6.0, and shake culture was carried out at 30°c for 60 hours. Cells were harvested from such culture fluids by using a centrifuge, then suspended in 100 mM phosphate buffer (pH 6.5) and untrasonically disrupted to give a cell-free exract.

In the Claims:

3. (Amended) The polypeptide according to Claim 1 [or 2] which is derived from a microorganism belonging to the genus Micrococcus.

5. (Amended) A DNA coding for the polypeptide according to [any of Claims 1 to 4] Claim 1.

8. (Amended) An expression vector containing DNAs according to [any of Claims 5 to 7] Claim 1.

13. (Amended) A transformant containing the expression vector according to [any of Claims 8 to 12] Claim 1.

14. (Amended) A transformant containing both the expression vector according to Claim [8 or 9] 1 and an expression vector containing a DNA coding for a polypeptide having glucose dehydrogenase.

16. (Amended) The transformant according to [any of Claims 13 to 15] Claim 1, wherein a host thereof is Escherichia coli.

20. (Amended) A production method of (S)-N-benzyl-3-pyrrolidinol comprising

a step of reacting the transformant according to [any of Claims 13 to 19] Claim 1 and/or a treated product thereof with N-benzyl-3-pyrrolidinone, and

a step of harvesting the thus-produced (S)-N-benzyl-3-pyrrolidinol.